AD_____

Award Number: DAMD17-00-1-0369

TITLE: Synthesis of Selective Inhibitors of 17β-Hydroxysteroid Dehydrogenase

PRINCIPAL INVESTIGATOR: Jeremy P. Barlow
David L. Vander Jagt, Ph.D.

CONTRACTING ORGANIZATION: University of New Mexico
Albuquerque, New Mexico 87131

REPORT DATE: July 2002

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | July 2002 | Annual Summary (1 Jul 01 - 30 Jun 02) |

**4. TITLE AND SUBTITLE**
Synthesis of Selective Inhibitors of 17β-Hydroxysteroid Dehydrogenase

**5. FUNDING NUMBERS**
DAMD17-00-1-0369

**6. AUTHOR(S)**
Jeremy P. Barlow
David L. Vander Jagt, Ph.D.

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
University of New Mexico
Albuquerque, New Mexico 87131

E-Mail: jbarlow@sandia.net

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. Abstract** ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Estradiol works at the level of the genetic material of the breast epithelial cells to control a wide range of genes that determine how fast the cell will grow. Breast cancer cells often remain sensitive to estradiol subsequent to becoming cancer cells. Type I 17β-hydroxysteroid dehydrogenase (HSD) is the enzyme responsible for reducing the hormone estrone to estradiol in the epithelial cells of the breast. In many cases of breast cancer, elevated quantities of HSD have been observed associating it with abnormal cell proliferation. It has therefore become our task to try and inhibit HSD's catalytic function. We have discovered that dihydroxynaphthoic acids inhibit dehydrogenase enzymes, and we also know how to design variations among this class of inhibitors. With intentions of finding a new cancer therapeutic, it has been our goal to utilize structure-based drug design and molecular modeling to develop selective inhibitors of human HSD and to test these for activity against human breast cancer cells grown in culture.

| 14. SUBJECT TERMS | 15. NUMBER OF PAGES |
|---|---|
| breast cancer, structure-based drug design, 17β-hydroxysteroid dehydrogenase, combinatorial chemistry, Rossmann fold, synthetic chemistry | 13 |
| | 16. PRICE CODE |

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

Table of Contents

## Introduction:

Hydroxysteroid dehydrogenases (HSD) play an essential role in the biosynthesis and degradation of steroid hormones. 17β-HSDs are a subclass of HSD isoenzymes that specifically participate in the final steps of the synthesis of estrogens and androgens. Human type 1 17β-HSD, also known as 17β-estradiol dehydrogenase, catalyzes the reduction of estrone to 17β-estradiol, the biologically active estrogen involved in the development of human breast cancer[1]. Since 17β-estradiol controls cell proliferation via ligand occupancy of the nuclear estrogen receptor, 17β-HSD and its associated activation of this steroid, has become the key enzyme associated with tumor cell proliferation[1,2]. Type-1 17β-HSD can, therefore, be viewed as a molecular switch. As this enzyme is present in elevated concentrations of hormone-sensitive breast cancer cell lines a compelling case can be made for the development of selective inhibitors of 17β-HSD as an approach to the design of new therapeutics in the treatment of breast cancer. We have recently developed a new class of dehydrogenase inhibitors that are targeted at the NAD(P)/NAD(P)H binding sites of dehydrogenases[1,2]. Collectively, these binding sites contain a specific three dimensional array of parallel beta strands and associated alpha helices that are known as the dinucleotide or Rossmann fold. Surprisingly, these inhibitors exhibit selectivity for different dehydrogenases based on slight variants in the amino acid moieties of their Rossmann folds[3,4]. The goal of this project is to develop selective inhibitors of Type 1 17β-HSD as "lead compounds" for structure-based drug design using the novel concept of targeting the Rossmann fold for inhibition.

## Body:

In the statement of work, the following tasks were proposed:

**Task 1**

The development of synthetic schemes for preparation of mono- and dihydroxynaphthoic acids as potential inhibitors of 17β-HSD-1 (months 1-36, an ongoing activity). New synthetic methodologies will be explored focusing on synthon development towards more efficient convergent syntheses. Using an optimized convergent synthesis, a higher number of variant substituted synthons can be made and used in the same time frame. In this way more target compounds can be made in the same period, and with higher overall yields.

**Task 2**

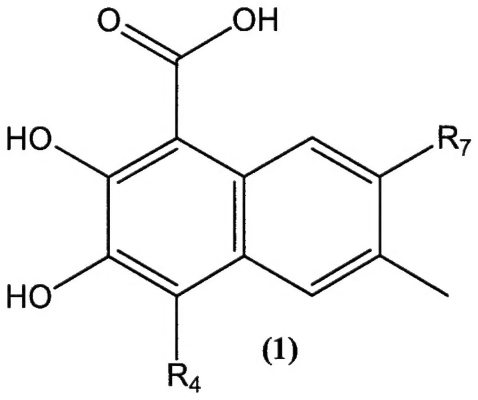
The development of combinatorial libraries (months 1-36, an ongoing activity). New and old synthetic methodologies will be combined for combinatorial syntheses of specific libraries for higher thru put activity screening against different dehydrogenases. These methods lend themselves to parallel synthesis particularly well.

**Task 3**

The development of Pan-Active-Site inhibitors, directed by molecular modeling and kinetic results (months 1-36, an ongoing activity). Information from modeling and screening of libraries will be used in conjunction to probe the mechanism of inhibition as well as to design next generation inhibitors.

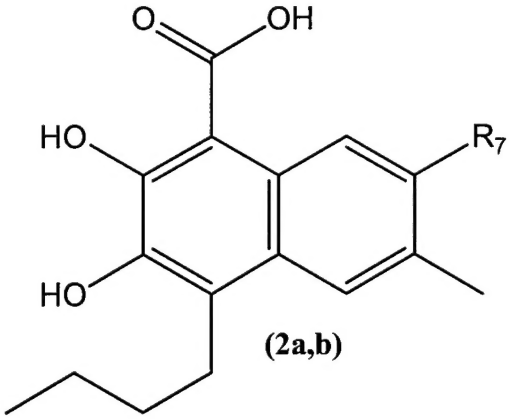
Following developments made to our synthetic scheme last summer, two new dihydroxynaphthoic acids have been synthesized in which an *n*-butyl group has been placed in the four position with a proton and a methyl group occupying the seven position respectively (see figure 1, compounds **2a,b**).

**Figure 1**




**(1)**

General naphthoic acid inhibitor




**(2a,b)**

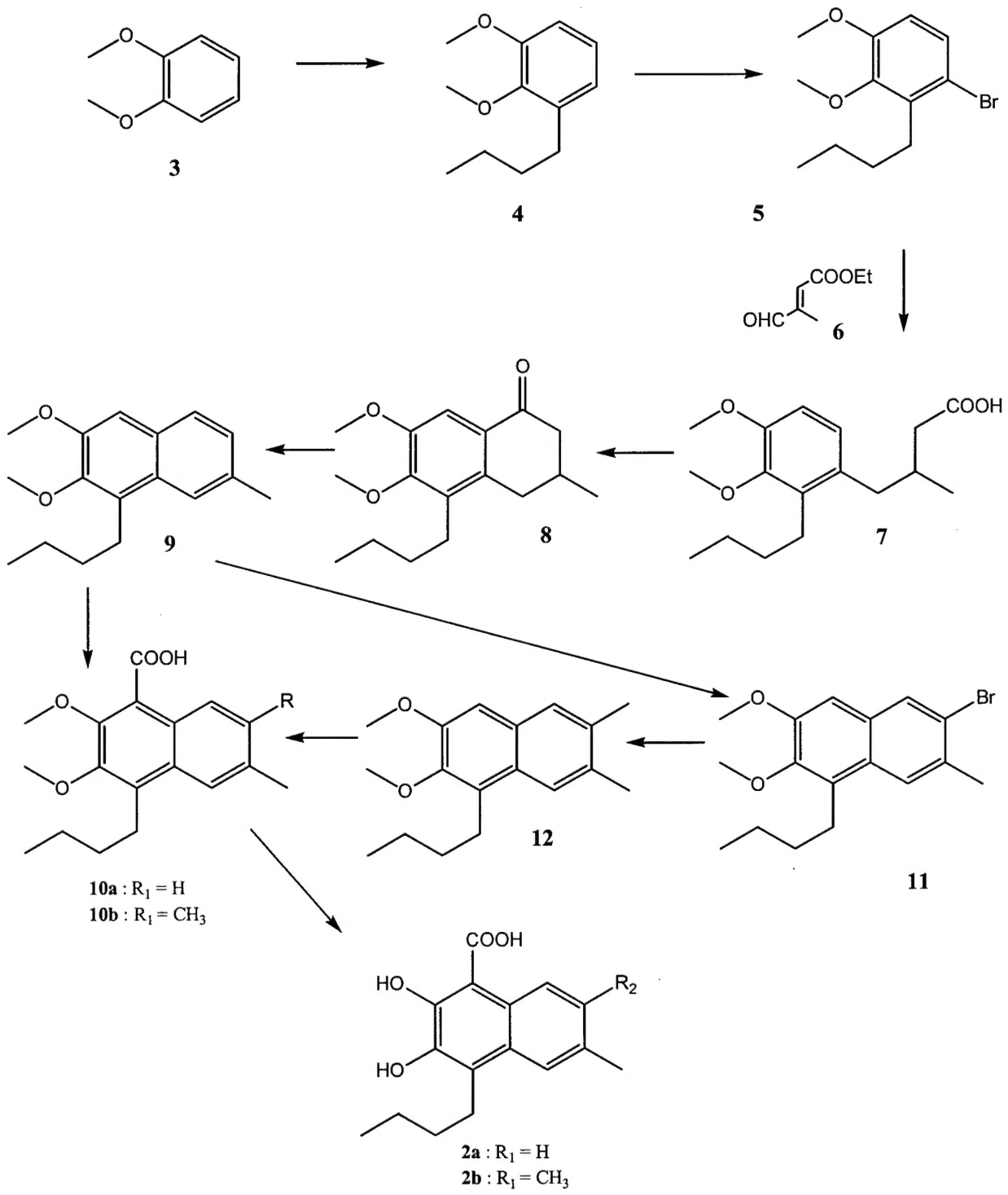
Two latest inhibitors: $R_7$ = H; $CH_3$

This was accomplished using scheme 1 in which veratrole (compound **3**) was used as a starting material and reacted with *n*-butyl lithium and butyraldehyde. The resulting benzilic alcohol was hydrogenolyzed with Pd/C and $H_2$ and treated with bromine at -10° C to give compound **5**. The bromide was then reacted with magnesium to afford the Grignard reagent which was reacted with ester aldehyde (compound **6**) to afford an alcohol. Saponification of the ester followed by catalytic reduction of the alkene and benzilic hydroxyl group yielded compound **7**. The acid was then cyclized using polyphosphoric ester (PPE) to give the tetralone (**8**). The tetralone was reduced with sodium borohydride to give the alcohol which was subsequently dehydrated to give the alkene (**9**). The alkene was then oxidized using DDQ and formylated using titanium tetrachloride and dichloromethyl methyl ether. The formyl group was then oxidized to an acid using sodium chlorite and peroxide to give compound **10**. The inhibitor (**2a**) is then afforded by a boron tribromide induced hydrolysis of the phenolic ethers. Likewise, compound **2b** was formed via bromination of the alkene (**9**) followed by a dehydrohalogenation reaction using dimethyl formamide to give compound **11**. A subsequent metallation reaction using n-butlylithium followed by the addition of methyl iodide yielded alkylated alkene. Oxidation to the naphthalene system (**12**) and the addition of the acid group was performed synonymously to the formation of **2a**. Subsequent hydrolysis of the phenolic ethers yielded inhibitor **2b**.

Although the larger butyl group in the four position (relative to propyl, ethyl and methyl groups of past inhibitors) was thought to exhibit potential to exert pan-active inhibition on 17β-HSD, the resulting $K_i$ values derived from these compounds were mediocre. However, encouraging modeling studies recently performed by Mike Brown and myself are demonstrating that the orientation of naphthoic acids in the active site of 17β-HSD is not conducive to pan-active inhibition. Rather, we have found that naphthoic acids arrange themselves with the acid group pointing towards the substrate binding domain while groups in the four position of these compounds exhibit interactions with residues normally associated with the recognition of the

nicotinamide ribose ring of NADPH. Interpretation of these results point towards the lack of occupation of the substrate binding site by the butyl group and, therefore, the diminished

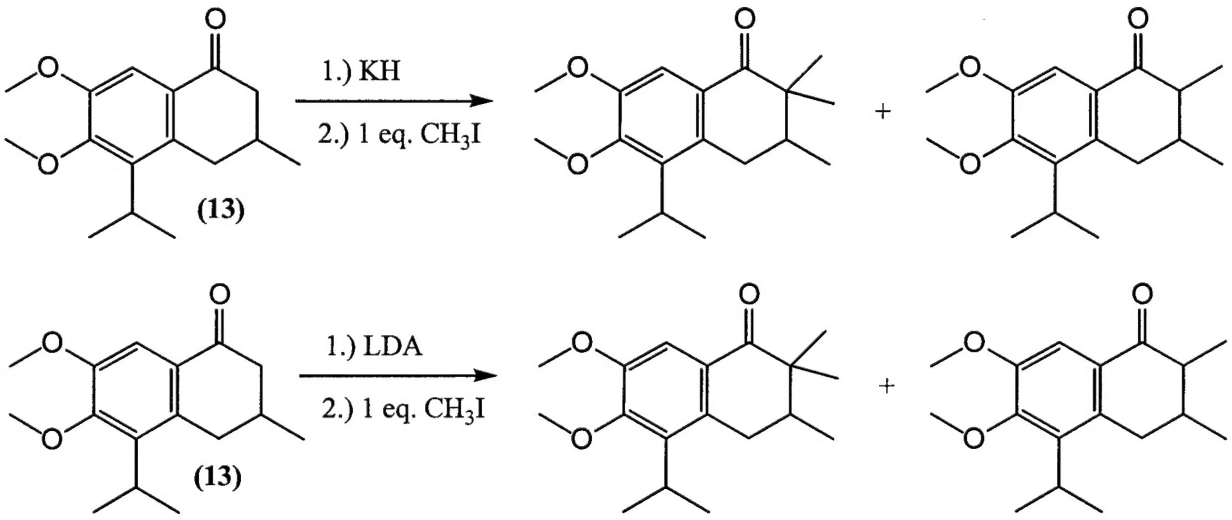
**Scheme 1**



inhibition associated with these compounds. However, our best modeling results are still being derived from the use of the lactone and iminolactone derivatives of monomeric gossypol in which groups in the four and seven positions exhibit the propensity to infringe upon both the substrate binding site and the adenine ring domain of the cofactor binding site respectively. The occupation of these sites combined with occupation of the nicotinamide binding portion of the cofactor binding site should result in the pan-active inhibition mentioned previously.

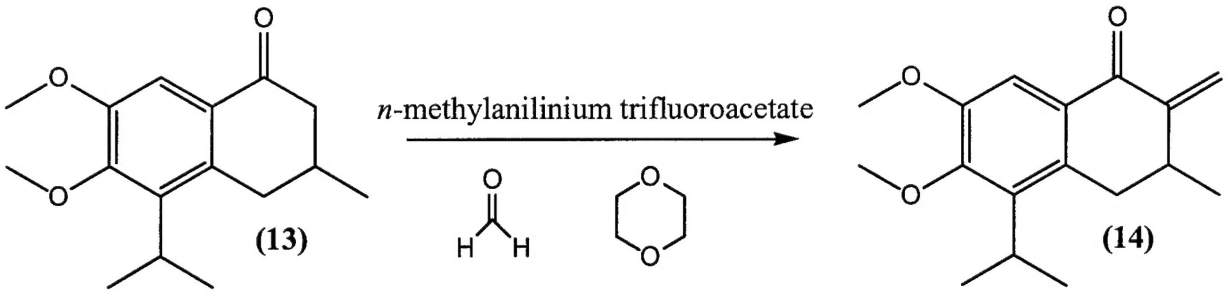
Last summer we reported a proposed scheme to synthesize a series of monomeric lactone and iminolactone derivatives of gossypol, and we have since started to synthesize these compounds with an isopropyl group in the four position (target compounds **27** and **28**). Since that time the synthetic scheme has been revised to alleviate complications within some reactions. Previously, we reported on the use of potassium hydride and alkyl halides for the alkylation of our tetralones (**13**) in the seven position. This past year's work has shown, however, that these reactions impart a lack of control in the alkylation process. Typical products from reactions of this type include a high percentage polyalkylated product, starting material, and the desired product in low yields. The low yields and difficulties in separating polyalkylated products from the desired, monoalkylated products have resulted in attempts at other alkylation reactions. Similarly, we have tried these reactions using LDA and alkyl halides with mixed results congruent to the potassium hydride reactions (see figure 2).

**Figure 2**



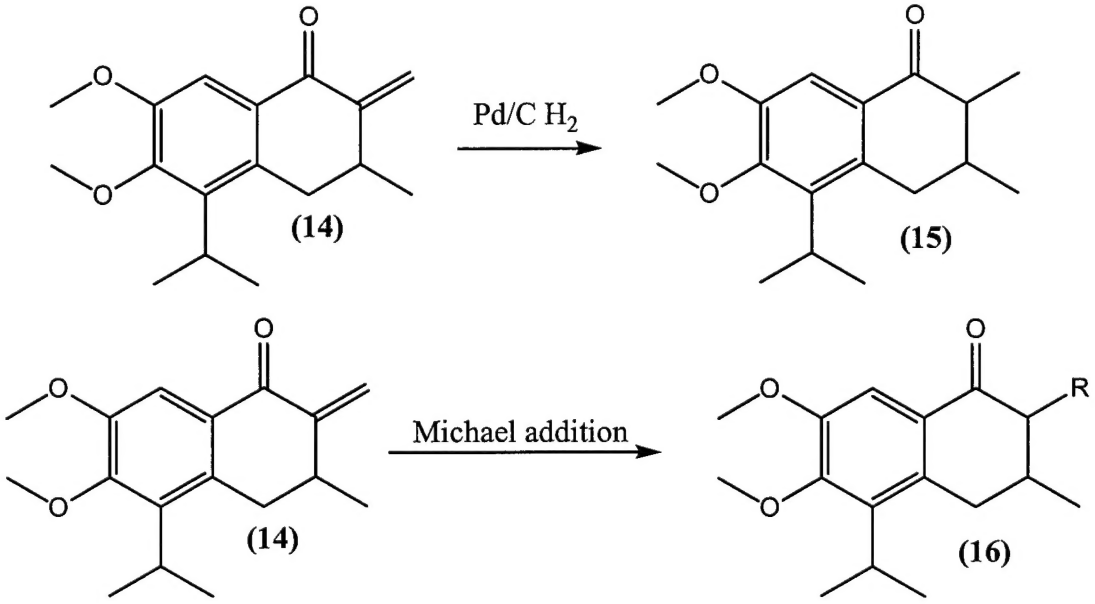

The new scheme developed to circumvent these problems involves the synthesis of an exocyclic methylene tetralone by treating compound **13** with *n*-methylanilinium trifluoroacetate and paraformaldehyde using dioxane as a solvent[5]. The reaction is refluxed under nitrogen for approximately 48 hours to afford yields on the order of ninety percent (see scheme 2).

**Scheme 2**



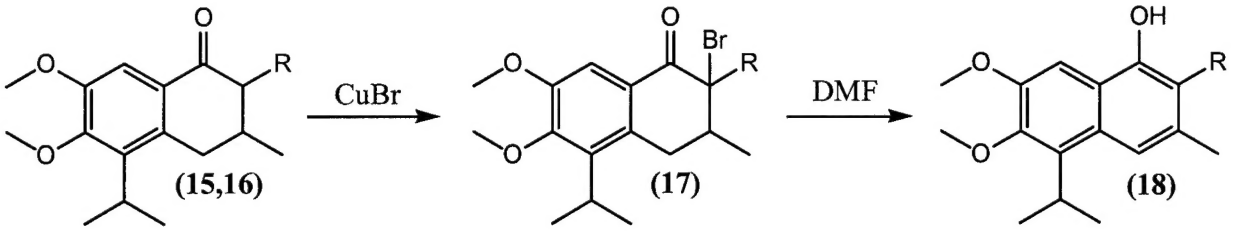

The resulting α,β unsaturated ketone (compound **14**) lends itself to not only catalytic reduction of the alkene for simple methyl substituents in the seven position (compound **15**), but to Michael addition chemistry for alternate, larger groups and combinatorial synthesis (compound **16**; see scheme 3).

**Scheme 3**

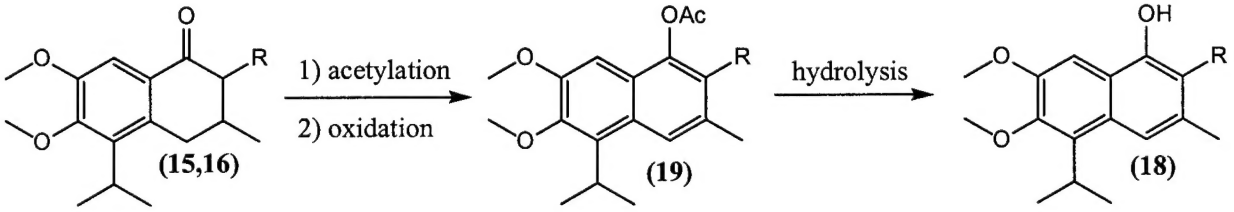



Moreover, because two alternate routes to the target compounds may now be exploited, this new scheme exhibits built-in diversity. The synthesis can either continue through a selective alpha bromination using cuprous bromide followed by a DMF induced dehydrohalogenation reaction with isomerization to the phenol (scheme 4), or it may be continued through an enol acetate derivative followed by an oxidation to complete the naphthalene system. Subsequent hydrolysis of the acetate results in the convergence of these two schemes at the phenol (compound **18**; scheme 5 & 6) where all subsequent chemistry has been performed previously[2,4].

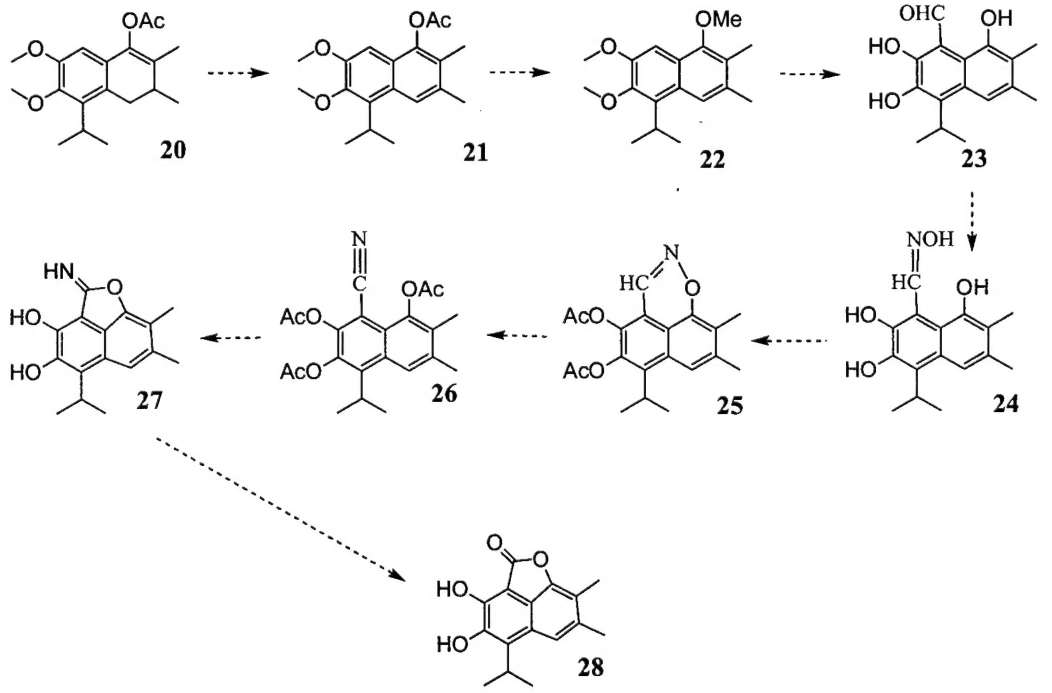
**Scheme 4**




**Scheme 5**



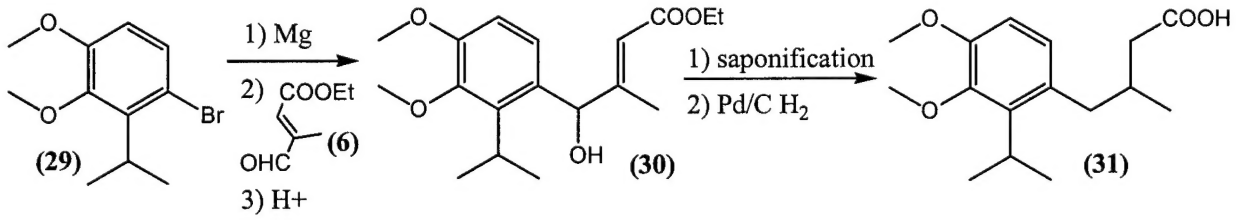

**Scheme 6**



The substituted tetralones (**18**) may then be treated with isopropenyl acetate and *p*-toluene sulfonic acid to form enol acetates **20**. The enol acetates could then be treated with DDQ to yield phenolic acetates, although stirring in open air should suffice for oxidation to give **21.** Compound **21** may then be hydrolyzed to the corresponding phenols and methylated with potassium carbonate and dimethylsulfate to form trimethoxynaphthalenes **22**. Formylation and hydrolysis of the phenolic ethers of **22** will also be accomplished by the use of *t*-butyllithium and DMF followed by the boron tribromide induced hydrolysis of the methoxy groups to afford compound **23**[6]. Compound **24** can be made by treating **23** with neutralized hydroxylamine hydrochloride in methanol[4]. Compound **24** can be cyclized to an oxime by acetylation of the phenolic ethers with acetic anhydride followed by the addition of sodium acetate[4]. Gradual heating on a boiling water bath completes the cyclization reaction to yield oxime **25**[4]. Although the isolation of **25** may be done for structural determination and reaction confirmation reasons, compound **26** may be prepared by subsequent reflux of **25** without isolation[4]. The iminolactone **27** can be prepared from **26** via a two step reaction[4]. The nitrile hexaacetate is first treated with potassium carbonate, water, and methanol to give the 1,1'-diacetate which may be cyclized to the iminolactone by refluxing with sulfuric acid. Hemigossylic lactones **28** can be obtained via reflux of their iminolactone equivalents in HCl and ethanol[4]. This scheme also allows for the introduction of nitrile derivatives for further study in that all acetate groups may be hydrolyzed from **26** (see scheme 6)[4].

Last year, I also reported on work being done with respect to synthon chemistry (compound **6**). I reported that because reduction of the alkene functionality combined with the hydrogenolysis of the hydroxyl group resulting from the Grignard addition (compound **30**) is a process that tends to lower the yields of the synthetic scheme (see scheme 7), we were working on alternate methods to circumvent this problem.

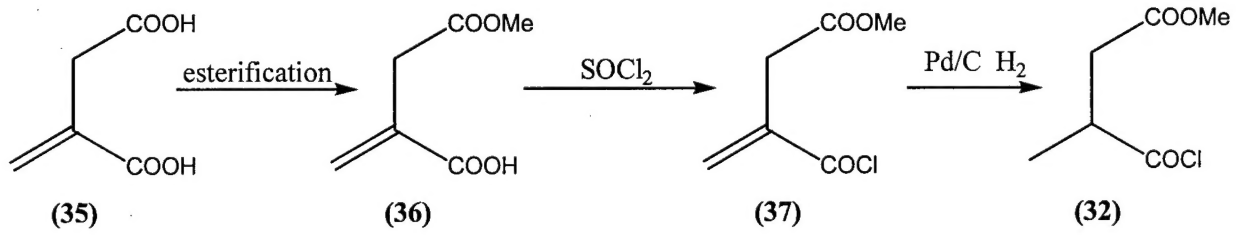
**Scheme 7**




I reported that attempts were being made to saturate the synthon prior to the addition reaction to improve our yields by rendering hydrogenolysis the only necessary subsequent step. We have found, however, that the ester and aldehyde functionalities of the synthon are complicating the catalytic reduction and have since been trying to synthesize the synthon in its saturated, acyl halide form. DR. Royer has found that the synthesis can begin with itaconic acid which may be selectively esterified with methanol and acetyl chloride followed by halogenation with thionyl chloride (see scheme 8). The resulting acyl halide (compound **37**) is reduced catalytically and

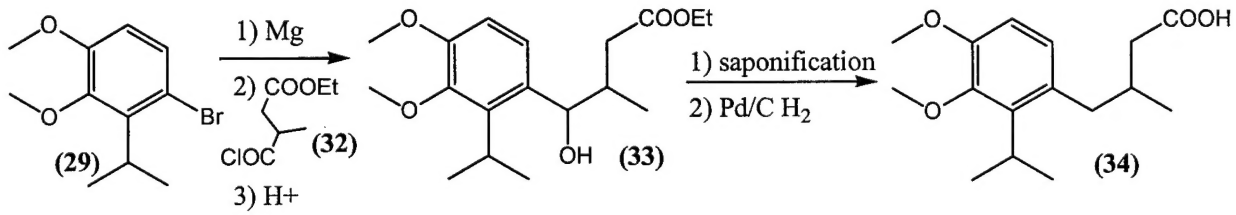
combined with the parent compound (compound **29**) in a Grignard reaction to yield the product in its saturated form (see scheme 9).

**Scheme 8**




At the moment our yields are adequate though we are confident they can be improved with further study. Furthermore, it is our hypothesis that the itaconic acid may be substituted for similar acids with alternate functionality.

**Scheme 9**




If successful, substituents in the seven position may not only be added by Michael chemistry, but by the use of different synthons as well. Moreover, such diversification will allow for the placement of alternate functional groups in the six position allowing for further insight into the mechanism of inactivation of 17β-HSD.

**Key research accomplishments:**

- Synthetic scheme has been made more convergent, efficient and diverse
- Synthetic scheme has added potential for the diversity of R groups in four, six and seven positions
- Increased diversity for the addition of R groups to naphthalene system
- The introduction of two possible schemes to synthesize target compounds should adversity be encountered with a particular scheme
-The completed synthesis of two naphthoic acid inhibitors with butyl in four position and their characterization as inhibitors
- Use of molecular modeling to determine that hemigossylic lactone and imino lactone molecules show more potential for pan-active inhibition than naphthoic acids
- Introduction of chemistry to the synthetic schemes allowing for combinatorial synthesis

**Conclusion:**

Our work over the past year has given great understanding of the types of compounds necessary to create a pan-active inhibitor of 17β-HSD. After completing the total synthesis of two naphthoic acids inhibitors and evaluating their characteristic inhibition constants for 17β-HSD we have found that this class of compounds (while exhibiting moderate inhibition) could be greatly improved. After further modeling studies, it was determined that naphthoic acids retain a different orientation in the Rossmann fold relative to other inhibitors. It was also discovered that the orientation of hemigossypol derivatives such as hemigossylic lactone and iminolactone show increased potential for pan-active inhibition relative to their naphthoic acid counterparts. This new insight led us to not only begin the synthesis of hemigossylic lactones and iminolactones, but to further develop the scheme to allow for variety in the location and type of possible inhibitors. Our new scheme also allows for development of combinatorial chemistry and associated libraries. Furthermore, new chemistry with respect to the synthesis of exocyclic methylene tetralones and their associated reductions opened new avenues in our synthetic scheme allowing for further diversity. In addition, our scheme allows for the development of two inhibitors at once as any iminolactone inhibitor is easily converted to its lactone counterpart in a single-stepped, facile imine hydrolysis. Furthermore, an intermediate in our scheme includes nitrile derivatives of these compounds as well. If molecular modeling suggests that nitrile compounds could generate pan-active inhibition, these compounds are easily synthesized without further modifications to our scheme. Thus we have seen that completing this synthetic scheme once can produce three different inhibitors.

Once a battery of inhibitors has been created, molecular modeling will allow us to develop a pharmacaphore for our active site, which, in turn, gives us more insight into the type(s) of compounds we should be making. Overall, the potential to develop at least three new inhibitors over the next year exists, allowing us a better understanding into the mechanism of inactivation of 17β-HSD.

**References:**

1. MS Gomez, RC Piper, LA. Hunsaker, RE Royer, LM Deck, MT Makler and DL Vander Jagt (1997), Substrate and cofactor specificity and selective inhibition of lactate dehydrogenase from the malarial parasite *P. falciparum*. **Mol Biochem Parasitol 90**, 235-246.

2. LM Deck, RE Royer, BB Chamblee, VM Hernandez, RR Malone, JE Torres, LA Hunsaker, RC Piper, MT Makler and DL Vander Jagt (1998) Selective inhibitors of human lactate dehydrogenases and lactate dehydrogenase from the malarial parasite *Plasmodium falciparum*. **J Med Chem 41**, 3879-3887.

3. JE Torres, J Deck, K Caprihan, LA Hunsaker, LM Deck and DL Vander Jagt (1998) Dihydroxynaphthoic acids: a new class of active site dehydrogenase inhibitors. **FASEB J 12**, 271A.

4. RE Royer, LM Deck, NM Campos, LA Hunsaker and DL Vander Jagt (1986) Biologically active derivatives of gossypol: synthesis and antimalarial activities of peri-acylated gossylic nitriles **J Med Chem 29**, 1799-1801.

5. J.L. Gras (1980) Methylene ketones and aldehydes by simple, direct methylene transfer: 2-methylene-1-oxo-1,2,3,4-tetrahydronaphthalene **Organic Synthesis 60**, 88-91

6. Manmade A.; Herlihj P.; Quick J.; Duffley R. P.; Hoffer A. P. (1983) Gossypol: Synthesis and in vitro spermicidal activity of isomeric hemigossypol derivatives **Experientia**, 1276-1277